

Allergenic Potential of Tomatoes Cultivated in Organic and Conventional Systems

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Abstract Tomatoes (*Lycopersicon esculentum* Mill.) are a widely consumed vegetables and contain many health beneficial micronutrients. Unfortunately, they may also cause adverse allergic reactions in sensitized people. Many studies, conducted in recent years, indicate that organically produced vegetables have higher nutritional value, improved sensory quality and contain more health-enhancing bioactive compounds than vegetables grown under the conventional system. However, the relation between organic methods of cultivation and allergenic potential of tomatoes has received little scientific attention. This study analyzed samples of five tomato cultivars taken from organic and conventional systems over three consecutive years. The content of profilin, Bet v 1 and lipid transfer protein (LTP) analogues in tomato samples was determined using an indirect ELISA assay. Substantial quantities of these proteins were found in certain cultivars across all three years of cultivation. On the basis of these findings, organically grown tomatoes appear to offer little advantage over conventionally cultivated plants in terms of reduced allergenic potential.

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Abbreviations

BSA	Bovine serum albumin
DBPCFC	Double-blind placebo-controlled food challenges
LTP	Lipid transfer protein
PFN	Profilin
PR	Pathogenesis-related protein

Introduction

As many studies have shown, regular consumption of tomatoes decreases the risk of prostate cancer and cardiovascular diseases [1, 2], due to their high levels of antioxidant free radical scavengers (e.g., lycopene and β -carotene) [3, 4]. However, while regular consumption of tomatoes can have positive health benefits, the fruit can also trigger the occurrence of allergy. The most effective form of treatment for individuals with a diagnosed allergy to tomatoes is to exclude the fruit from the diet, although this may be associated with reduced quality of life.

Food allergies affect approximately 3–4 % of adults and 5 % of children [5]. Tomato allergies are especially common in people with pollen allergies [6]. Prevalence of sensitization to tomato in Europe is ranging from 0.8 % in Iceland to 5.6 % in Germany [7]. It has been reported that tomato allergies affect 39.2 % of people sensitized to grass pollen and between 2.2 and 4.3 % of people allergic to latex [6]. The most frequent clinical responses to tomatoes are local symptoms in the oral mucosa, but tomatoes may also elicit systemic reactions, that can lead

to serious illnesses [8]. A case of tomato-induced anaphylactic shock has been also reported [9].

In recent years, there has been increased demand for food cultivated under organic farming methods. This may be due to declining consumer confidence in conventionally farmed foods. Organic production uses natural processes and rejects the use of chemical compounds such as plant protection products (pesticides), mineral fertilizers and plant growth regulators. Instead, natural fertilizers (manure, compost, green manure legumes) and natural methods of plant protection (plant extracts) are used. In the organic system, plants are more responsible for fighting against their own diseases and pests, and for this reason synthesize more compounds as natural pesticides (*i.e.*, polyphenols) and defense proteins [10], many of which (such as Bet v 1 and LTP) are common allergens [11, 12].

The tomato allergens confirmed and listed in the official WHO/IUIS list of allergens [13] are: profilin – Sola l 1 with a mass of 16 kDa, previous name Lyc e 1 [6, 14]; β -fructofuranosidase – Sola l 2 (50 kDa), previous name Lyc e 2 [15–17]; lipid transfer protein – nsLTP 1 – Sola l 3 (9 kDa), Lyc e 3 [4, 6]; Bet v 1-related protein – Sola l 4, previous name Lyc e 4 [18]; 19 kDa cyclophilin – Sola l 5; nsLTP2 – Sola l 6 (7 kDa), nsLTP 1 – Sola l 7 (12.5 kDa). All above mentioned allergens (apart from cyclophilin) are classified as food allergens, however Sola l 7 is missing from the list of allergens. Furthermore the unofficial Allergome database [19] provided other tomato allergens such as: Sola l chitinase with a mass of 31 kDa; Sola l glucanase (55 kDa); Sola l peroxidase (45 kDa); Sola l globulin 11S (30 kDa); Sola l PG – polygalacturonase (36 kDa); Sola l PME – pectinesterase (36 kDa); thaumatin like-protein – Sola l TLP (27 kDa); Sola l vicilin – 7S vicilin (65 kDa); Sola l ARP60S – ribosomal protein P2; Sola l SOD – superoxide dismutase (15.3 kDa). Cross-reactivity between tomato, potato and latex allergens has been demonstrated in children and adults with tomato allergies due to the presence of patatin with a mass of 44–46 kDa [20].

In this study, we set out to identify and determine the content of analogues to the most common panallergens, Bet v 1, profilin and LTP, in cultivars of tomato from both conventional

and organic farms. Lipid transfer proteins (Sola l 3, LTP) are the most stable in terms of their resistance to digestion and temperature, and may still conserve their conformational structure in processed tomato products. In contrast, Bet v 1 (Sola l 4) and profilin (Sola l 1) are heat-labile and susceptible to digestion allergens, and can often be tolerated in processed tomato products by patients who elicit symptoms following consumption of raw tomatoes [21]. The Bet v 1, profilin and LTP analogues may also trigger allergic cross-hypersensitivity in patients allergic to other foods as well as to pollens. In this study, we investigate whether the system of cultivation, organic or conventional, affects the allergenic potential of tomatoes, and so whether organically cultivated tomatoes might be recommended for consumers with allergies. The influence of the year of cultivation and genotype on allergen content is also examined.

Materials and Methods

Plant Materials The five tomato cultivars tested were Merkury, Akord, Rumba (standard tomatoes), Conchita and Picolino (cherry type tomatoes) from the growing seasons of 2007, 2008 and 2009. The tomato plants were grown under a cover in foil tunnels in three different conventional farms and three certified organic farms under similar climatic, soil and agrotechnical conditions (Online Resource 1 and 2). All cultivars of tomatoes from each of the experimental farms were collected at the same stage of ripeness (red-ripe). The tomato fruits were washed, cut into pieces, rapidly frozen, then lyophilized. After lyophilization, the fruits were ground in a laboratory mill (Bionovo, Legnica, PL) and stored at -80°C prior to analysis.

Preparation of Tomato Plant Extracts The samples were extracted using Tris-glycine (T-G, pH 8.3) with 0.05 M Tris and 0.33 M glycine. 0.25 g of each lyophilized specimen was mixed with 5 mL of the T-G buffer and incubated on a laboratory shaker (TTS 2, Yellow Line, IKA-Werke GmbH & Co. KG, Staufen, Germany) for 1 h. All samples were then

Fig. 1 The content of profilin analogues in the organic and conventional cultivars from the years 2007–2009 [AUG-1]

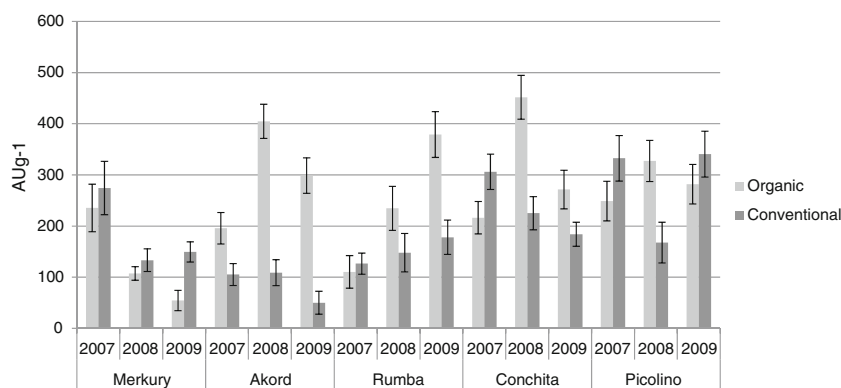
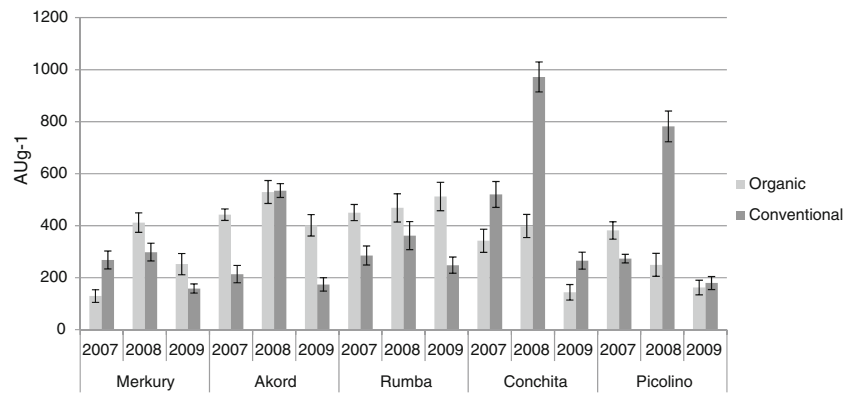


Fig. 2 The content of Bet v 1 analogues in the organic and conventional cultivars from the years 2007–2009 [AUg-1]



centrifuged (3500 rpm for 10 min) using a Sigma 2-16P centrifuge (Polygen, Wrocław, Poland) and the supernatants collected and dialysed against T-G buffer (pH 8.3) containing 3 mmol/L sodium azide using a molecular porous dialysis membrane (Spectra/Por 1, cutoff: 6–8 kDa and 3 kDa; Spectrum Laboratories Inc., Florida, USA). The dialysates were stored at -20°C prior to analysis. Protein concentration was determined using the Pierce method and a BCA Protein Assay Kit (Thermoscientific, Rockford, USA) with bovine serum albumin (BSA) as a standard.

Indirect ELISA Procedure 96-microwell ELISA plates (Immunoplate Maxisorp F96 certified; Nunc, Roskilde, Denmark) were coated overnight at 4°C using 100 μL of coating solution (diluted tomato extracts obtained from lyophilized samples of the different cultivars after mixing with 0.1 M carbonate-bicarbonate buffer pH = 9.6). The protein concentration was 2–5 $\mu\text{g}/\text{mL}$ for each well. The plates were then washed four times with a PBS-T washing buffer (pH = 7.4, PBS, 0.1 % Tween-20) and free binding sites were blocked by incubating the plates for 2 h at room temperature with 3 % solution of low fat milk in PBS-T buffer. The milk buffer solution was then removed and the plates rinsed four times with PBS-T. The plates were further incubated with 100 μL of diluted (1:1000) rabbit anti-profilin antibodies (Sigma-Aldrich, St. Louis, MO, USA) or with mouse (diluted 1:500) anti-Bet v 1 antibodies (Dendritics, Lyon,

France) and anti-LTP (Abcam, Cambridge, United Kingdom) antibodies, at room temperature for 1 h or 2 h, respectively. The plates were washed again and 100 μL of goat antibodies (diluted 1:5000) against rabbit (Sigma-Aldrich, St. Louis, MO, USA) or mouse IgG (Sigma-Aldrich, St. Louis, MO, USA) coupled to alkaline phosphatase (AP) added. After incubation with secondary antibodies for 1 h at room temperature, the wells were washed four times with PBS-T. 100 μL of the phosphatase substrate p-NPP (Sigma-Aldrich, St. Louis, MO, USA) were then added to each well and incubated for 0.5 h. The reaction was terminated by adding 100 μL of 3 M NaOH solution and the absorbance measured using a Multiscan RC reader (ThermoLabsystem, FL) at 405 nm. The optical density (OD) of a control well without primary antibodies was used to assess background. The Bet v 1, profilin and LTP analogue content was expressed as a unit of standard allergen (AU) per 1 g of sample. The standard allergens used, rPru p 1, rPru p 4 and rPru p 3, were recombinant allergens from peach (*Prunus persica*), equivalent to Bet v 1, profilin and LTP, respectively.

Statistical Analysis

Statistical calculations were carried out using two-way analysis of variance with the use of the Tukey's test ($\alpha = 0.05$). Lack of statistically significant differences between groups

Fig. 3 The content of LTP analogues in the organic and conventional cultivars from the years 2007–2009 [AUg-1]

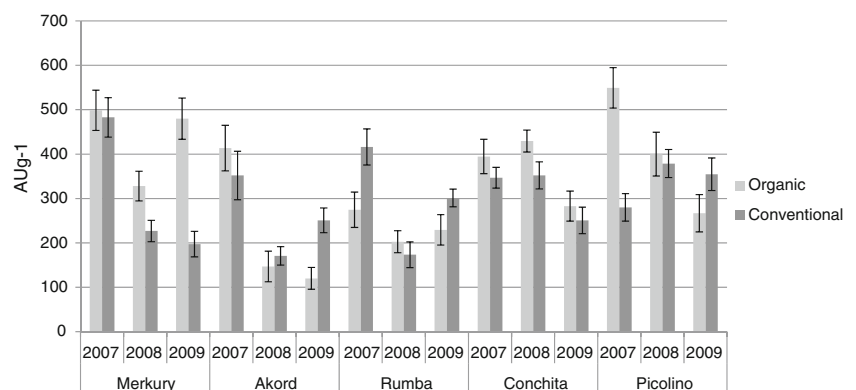


Table 1 The average content of allergens in dependence on cultivation method [AUG-1]

	Count ^c	Bet v 1			LTP			Profilin		
		Average	SD ^a	Standard error	Average	SD	Standard error	Average	SD	Standard error
Organic tomatoes	45	348.2a ^b	124.6	71.9	334.4a	125.6	72.7	254.5b	107.5	62.1
Conv. tomatoes	45	369.0a	225.9	130.4	302.2a	88.5	51.1	188.6a	85.4	49.3

^a SD, standard deviation. ^b Means in a column followed by the same letter (a, b) are not significantly different at the 5 % level of probability ($\alpha = 0.05$).

^c Count, the number of measurements used in the calculation of mean

examined was determined by the same letters. *P*-values were calculated with Statgraphics 5.1 software (StatPoint Technologies, Inc., Warrenton, VA, USA). The total number of measurements was 90. Each measurement was performed in triplicate ($n = 3$) and the average value of absorbance estimated for the sample solution.

Results and Discussion

The profilin, Bet v 1 and LTP analogue content was determined in five cultivars of tomato from both organic and conventional farms. The figures (Figs. 1, 2, and 3) below show the quantities of the potential allergens that exist in organically and conventionally cultivated tomatoes over three growing seasons: 2007, 2008 and 2009.

Profilin analogues in the range from 451.7 to 50.0 AUG-1 were found in each of the tested samples (Fig. 1). Allergenic protein content varied depending on the cultivar of the fruit. The highest profilin analogue content was found in the organic cultivars Conchita and Akord from 2008, in ranges between 451.7 ± 42.8 and 404.6 ± 33.4 AUG-1, respectively, while the lowest were found in a conventional cultivar of Akord from 2009 (50.0 ± 22.2). The conventionally grown cultivar of Merkury showed higher values in comparison to organic Merkury tomatoes in all three years of cultivation. In contrast, the organically grown Akord cultivar was characterized by higher profilin analogue content in all three years, with an up to fourfold difference between allergen levels in conventionally and organically grown cultivars. Similar results were found with Rumba and Conchita cultivars from the years 2008

and 2009, with allergen levels several times higher in organic in comparison to conventionally grown tomatoes. Profilin analogue content was higher in conventionally grown Picolino tomatoes from both 2007 and 2009, although a higher value was noted in organically grown tomatoes of this cultivar from 2008.

The content of Bet v 1 homologues ranged from 972.0 to 129.4 AUG-1, with the highest value observed in conventional cultivars of Conchita (972.0 ± 57.8) and Picolino (781.8 ± 59.4) from 2008, and the lowest value in organic Merkury tomatoes from 2007 (129.4 ± 24.5) (Fig. 2). Bet v 1 homologue content was found to be higher in organic tomato cultivars of Rumba from all three years of cultivation, in organic Merkury from the years 2008–2009 and in organic Akord from 2007 and 2009. However, it was also higher in the conventionally grown tomato cultivars Conchita (all three years of cultivations) and Picolino from the years 2008–2009, in comparison to organically grown tomatoes of the same cultivars. There is thus a clear relationship between the year of cultivation and variability in the amount of allergens in each tomato cultivar, while organic tomatoes were found to contain more allergens than conventionally grown tomatoes in most years.

A similar analysis of LTP analogues was conducted (Fig. 3), with levels found to range from 549.3 ± 45.6 for the organic cultivar of Picolino from the year 2007 to 120.0 ± 24.6 for the organic cultivar of Akord from 2009. As can be seen from the results, the amount of LTP homologues in the organically cultivated tomatoes was several times higher than in the conventionally farmed cultivars of Merkury and Conchita (all three years of cultivation), as well

Table 2 The average content of allergens in dependence on cultivation year [AUG-1]

	Count ^c	Bet v 1			LTP			Profilin		
		Average	SD ^a	Standard error	Average	SD	Standard error	Average	SD	Standard error
2007	30	330.7b ^b	113.5	65.5	400.8b	86.4	49.9	215.1a	76.4	44.1
2008	30	496.7c	207.9	120.5	280.8a	101.9	58.8	230.9a	117.8	68.0
2009	30	248.5a	110.0	63.5	273.3a	90.8	52.4	218.7a	108.1	62.4

^a SD, standard deviation. ^b Means in a column followed by the same letter (a-c) are not significantly different at the 5 % level of probability ($\alpha = 0.05$).

^c Count, the number of measurements used in the calculation of mean

as in Akord from 2007 and Picolino from the years 2007–2008. However, conventionally grown Rumba from the years 2007 and 2009 and Akord from 2008 to 2009 exhibited higher LTP analogue contents.

The statistical analysis was performed and the results are presented in Tables 1, 2, 3, and 4. It is shown, that the dependence between allergens content and the cultivar is statistically significant in case of Bet v 1 ($p = 0.00049$), LTP ($p < 0.0001$) and profilin ($p < 0.0001$). The cultivation year and connected with it weather conditions influence the content of Bet v 1 ($p < 0.0001$) and LTP ($p < 0.0001$) analogues. Only the profilin content reveals dependence on the method of cultivation ($p = 0.0008$) in all three years. Its content in organic tomatoes is higher.

Food products cultivated using conventional methods may contain harmful chemicals and often exhibit (toxic) allergenic properties, exemplified by sensitivity to pesticide residues, synthetic dyes, preservatives, sweeteners, etc. [22]. The most serious contaminants are pesticide residues, which may have genotoxic, carcinogenic, neurodestructive, endocrine and allergenic effects [23]. Foods produced by organic farming, which aims to achieve high quality without the use of artificial chemicals and fertilizers (or using only tolerable levels of these substances) and excludes the use of genetic modification techniques, should theoretically be less allergenic than those produced by conventional methods of industrial cultivation [23–25]. However, the results of this study find little support for the thesis that organic tomatoes contain less allergenic proteins than conventional.

Previous studies [10, 26, 27] of organic and non-organic tomatoes have focused on quality comparisons (nutritional, sensory values and bioactive compound content), with few addressing the issue of allergenicity [8, 28, 29]. Plants grown using conventional and organic systems differ in terms of their access to nutrients, which is reflected in their content of organic substances such as proteins. In a conventional system of cultivation employing soil fertilization, tomato plants absorb easily assimilable nitrogen from the substrate. The high concentration of this macroelement results in higher levels of

Table 4 P -value for allergens, method, year of cultivation and cultivar

	Bet v 1	LTP	Profilin
Method of cultivation	NS ^a	NS	0.0008
Year	<0.0001	<0.0001	NS
Cultivar	0.00049	<0.0001	0.0001

^a NS, not significant statistically

protein and protein compound synthesis, in comparison to tomatoes produced by organic farming [10].

Allergenicity studies on different cultivars of tomato have primarily involved skin prick tests and double-blind placebo-controlled food challenges (DBPCFC) on sensitized patients. Of the two tomato cultivars tested by Dölle et al. [28], an old landrace cultivar (Reisetomate) induced much lower levels of skin reaction and elicited far fewer symptoms after the oral challenge in comparison to an organic cultivar of Matina exhibiting the typical properties of resistance to environmental stress and disease [28]. In another work, Dölle et al. [8] demonstrated a correlation between positive skin allergy tests in tomato-allergic patients and both the genetic background and maturity level of tomato fruits. Skin reactions were much weaker with unripe green as opposed to ripe red tomatoes, and with landrace cultivars in comparison to cultivars grown for use in organic horticulture [8]. In both cases, skin tests confirmed the higher allergenic potential of organic cultivars, which was also found in most cases by our research. Nevertheless, studies involving patients have certain limitations, such as the intra-individual variability of results, as demonstrated by Bencivenni et al. [29], who have shown that the allergenic properties of 12 different ecotypes of tomato depend on the specific proteins to which the patient is allergic.

This study has found the differences in allergen content in the same tomato cultivars over three years of cultivation which may be due to variations in climatic conditions and the fact that natural stress conditions such as heat, cold and drought cause the synthesis of new proteins in plants. Other factors may include the cultivar/variety of plant selected for the study

Table 3 The average content of allergens in dependence on cultivar [AUG-1]

	Count ^c	Bet v 1			LTP			Profilin		
		Average	SD ^a	Standard error	Average	SD	Standard error	Average	SD	Standard error
Merkury	18	253.4a ^b	92.4	53.3	368.9b	124.8	72.1	159.0a	74.7	43.2
Akord	18	377.9bc	140.4	81.1	242.2a	108.5	62.6	193.8a	123.3	71.2
Rumba	18	382.8bc	87.8	50.7	266.2a	79.3	45.8	196.1a	91.1	52.6
Conchita	18	440.0c	260.2	150.2	342.7b	61.1	35.3	275.8a	87.9	50.8
Picolino	18	338.9ab	210.5	121.5	371.6b	93.2	53.8	283.1b	60.8	35.1

^a SD, standard deviation. ^b Means in a column followed by the same letter (a-c) are not significantly different at the 5 % level of probability ($\alpha = 0.05$).

^c Count, the number of measurements used in the calculation of mean

[30, 31], the fruit maturity status [8] and the length of storage [32]. Lopez-Matas et al. [30] found differences between the protein profiles, composition and quantity of allergens in six different cultivars of tomato. The dependence of allergenicity on plant cultivar was underlined in a study by Jensen-Jarolim et al. [33], who reported the presence of Bet v 1 protein (Cap a 4) in 50 % of the varieties of *Capsicum annuum* they analyzed. It can be concluded that the irregular expression of pathogenesis-related (PR) protein homologues in different cultivars of the same species is associated to their diverse resistance against microbial infections.

In many cases, organic cultivars show higher allergen contents in comparison to conventionally cultivated plants. Bet v 1 and LTP allergens belong to the family of pathogenesis-related proteins and, as a result, it may be concluded that different stress factors conduce the synthesis of these proteins in each of the cultivation systems. The higher levels of allergens from the PR family in organically grown plants can be explained by the fact that these plants are subject to biotic (fungi, viruses and bacteria) and abiotic stresses without the assistance from pesticides and fertilizers. Meanwhile, the chemicals used in non-organic cultivation can adversely affect the expression of the proteins by inhibiting the growth of microorganisms which are essential to PR protein synthesis. On the other hand, the high content of allergens from the PR family observed in some conventional cultivars may be a result of plant defense responses to such chemical agents. Similar conclusions were drawn in a study of apples by Matthes and Schmitz-Eiberger [32], in which the authors found lower content of Mal d 1 – Bet v 1 homologues in the majority of cultivars of organically grown fruit in comparison to apples grown using the integrated production method. These findings may be explained by the fact that the use of pesticides leads to a stronger response than biotic factors.

Conclusions

The demand for organic food continues to rise, driven largely by claims of its health value. However, this study finds little difference in terms of the allergenic potential of organic and non-organic tomatoes. Indeed, the content of panallergens is, in many cases, higher in organically farmed tomato cultivars, in comparison to the same cultivars grown conventionally. Due to cross-reactivity, this increases the risk of allergic reactions in people who are allergic not only to tomatoes, but also to pollen and other vegetables and fruits. Further studies are needed to confirm the allergenic potential of these cultivars of tomato in patients with tomato and pollen allergies, either by the use of skin prick tests, DBPCFC, or IgE-blotting. The content of other allergens in tomatoes grown under organic and conventional systems could also be compared, as well as the allergenicity of tomato-derived products. A long-term

study including other cultivars of tomato would eliminate the impact of seasonality.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human or Animal Studies This article does not contain any studies with human or animal subjects.

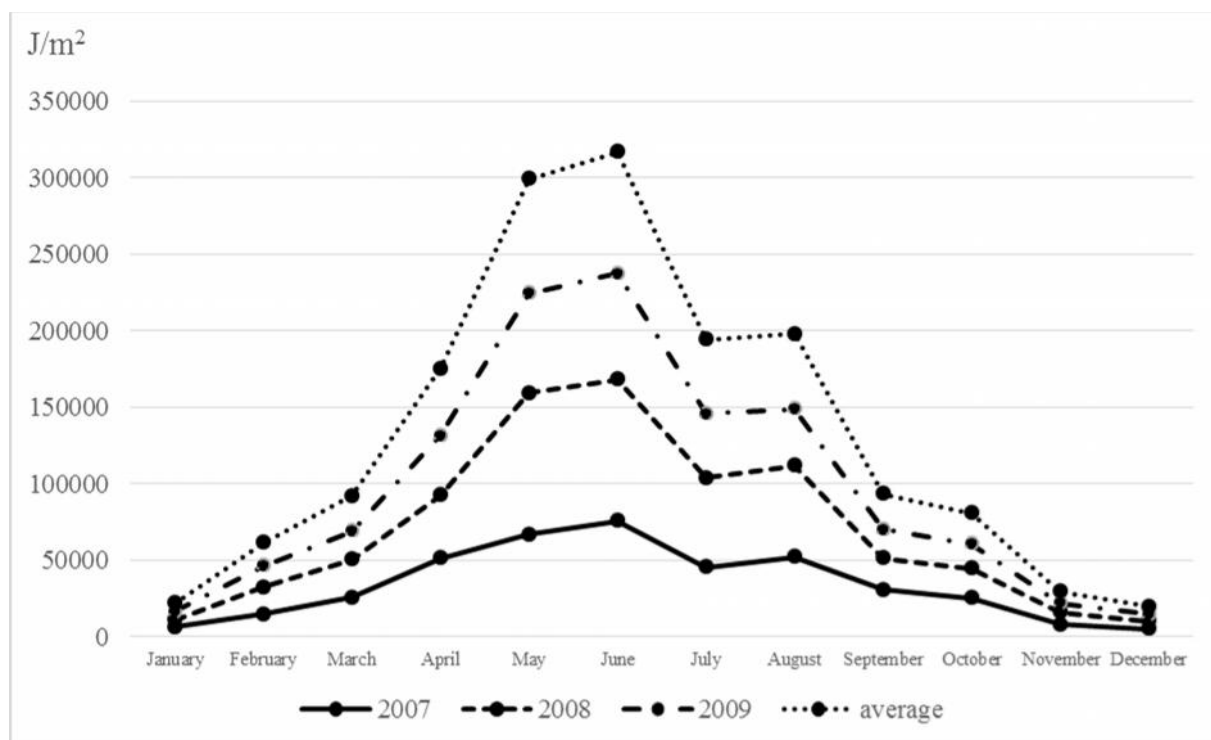
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Electronic supplementary material Table 1. Data about localization, fertilizers regime and dose, plant protection system and type of soil used in organic and conventional farms (average values for 2007-2009). Three organic and three conventional farms were located a very closely each other (see geographic localization data). Tomato plants were cultivated under plastic tunnels with surface area 350, 400 and 380 m² for organic as well as 450, 520 and 470 m² for conventional farms. One hundred and fifty plants were planted in every tunnel. During vegetation season the tomato plants were frequently watered by drip irrigation system. In every farms almost the same amount of water have been used. At the same time information about solar radiation intensity at the area of farms was collected in every experimental year and presented on Fig.1 (ESM_2).

Kind of farm	Localization	Fertilizers used	Type of soil	Plant protection system	Fertilization regime (kg/ha)				
					N	P	K	Mg	Ca
organic farm no. 1	Kaszewska Wola /51°30' N 20°55' E/	green manure (40 t/ha). Patentkali (P:S 30:10) basaltic flour. Humobak (mixture of bacteria and soil fungi)	sandy middle soil III category 15% (floatable particles) pH 6.7	Bioczos BR (garlic ekstrakt). Antifung 20 SL	140	30	165	100	120
organic farm no. 2	Radzanów /52°56' N 20°05' E/	compost 60%. Biohumus	sandy- loamy slight soil III category 15% (floatable particles) pH 6.9	Grevit 200 SL	150	45	160	90	130
organic farm no. 3	Sukowska Wola /51°29' N 20°57' E/	cow manure (8 t/ha)	sandy middle soil IV category 12% (floatable particles) pH 6.9	no protection	145	45	160	85	110
conventional farm no. 1	Kaszewska Wola /51°19' N 20°58' E/	cow manure (8 t ha ⁻¹). organic chalk, calcium and magnesium nitrate	sandy- loamy middle soil, III category 25% (floatable particles) pH 7.1	Sumilex 500 SC	230	110	160	50	130
conventional farm no. 1	Sewerynów /51°28' N 20°54' E/	Polidap (N:P:S 18:46:5). Grandar (N:P:K 3:9:19). Nitrosofosphate	sandy- clay slight soil III category 25% (floatable particles) pH 6.9	Mospilan 20 SP (acetamipiryde) Betoksan S 050 SL (2-naftoxyacetic acid)	220	120	140	35	130
conventional farm no. 3	Orońsko /51°18' N 20°59' E/	cow manure (0.5 t/ha) Patentkali (P:S 30:10) phosphate flour Polimag (N:P:K:Mg:S 10:8:15:5:4)	sandy middle soil III category 20% (floatable particles) pH 6.8	Miedzian 50 WP (copper). Biosept 33 SL (grapefruit extract)	250	140	180	40	140



Electronic supplementary material Fig. 1. The intensity of solar radiation (in J/m^2) on the area of experimental farms in 2007-2009. The information about solar radiation intensity was collected in every experimental year.